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(54) Assay for detecting HIV antigens and HIV antibodies

(57) There is described an assay to simultaneously detect the presence of antigen and antibody analytes of a human immunedeficiency virus (HIV) in a sample, comprising the steps of simultaneously or sequentially contacting the sample with
a) at least one antigen capture reagent which is an antibody or a fragment thereof specific for an epitope on a gag antigen analyte,
b) at least two antibody capture reagents wherein one antibody capture reagent comprises an epitope which is specific for an envelope antibody analyte, and one antibody capture reagent comprises an epitope which is specific for a capsid antibody analyte, and
c) detecting the antigen/antigen capture reagent and antibody/antibody capture reagent complexes, provided that the gag antigen analyte is not the capsid antigen.

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Figure 1

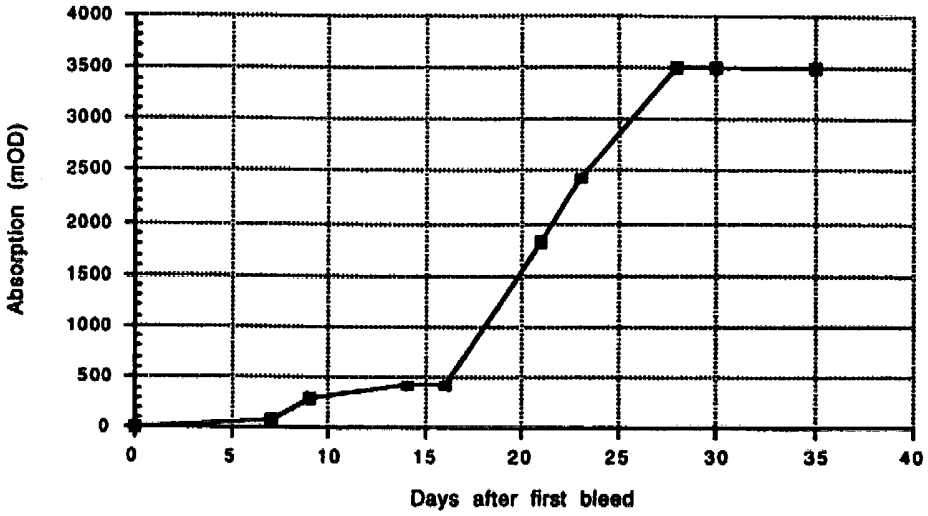


Figure 2

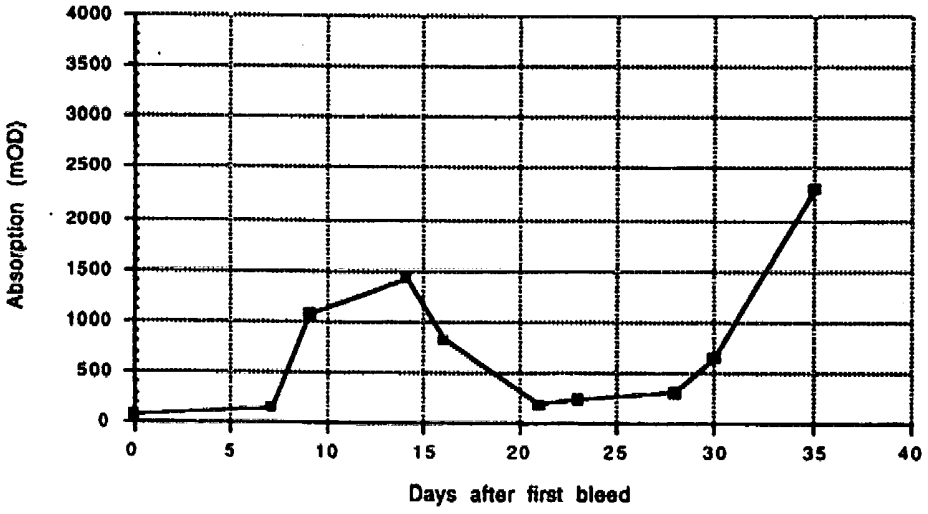


Figure 3

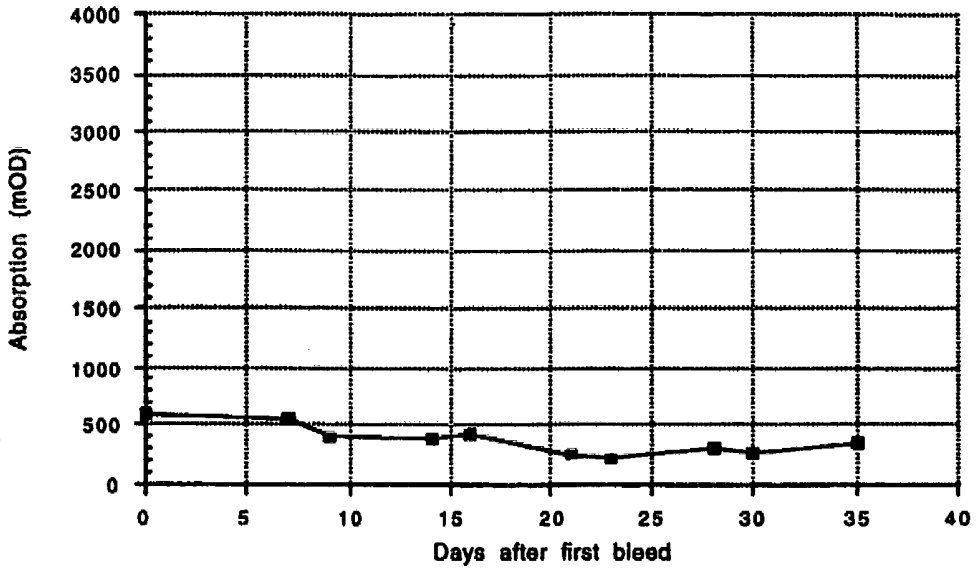


Figure 4

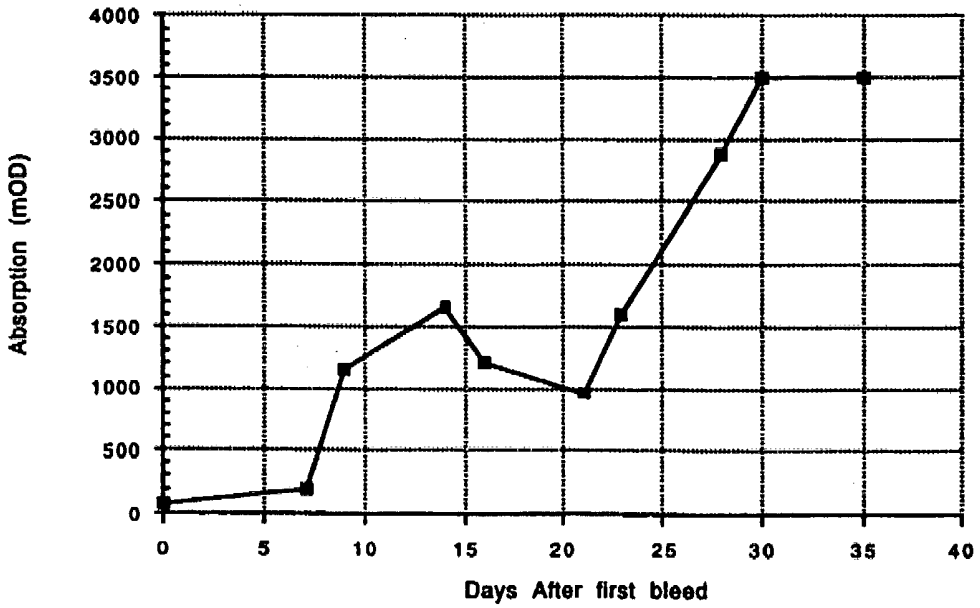


Figure 5

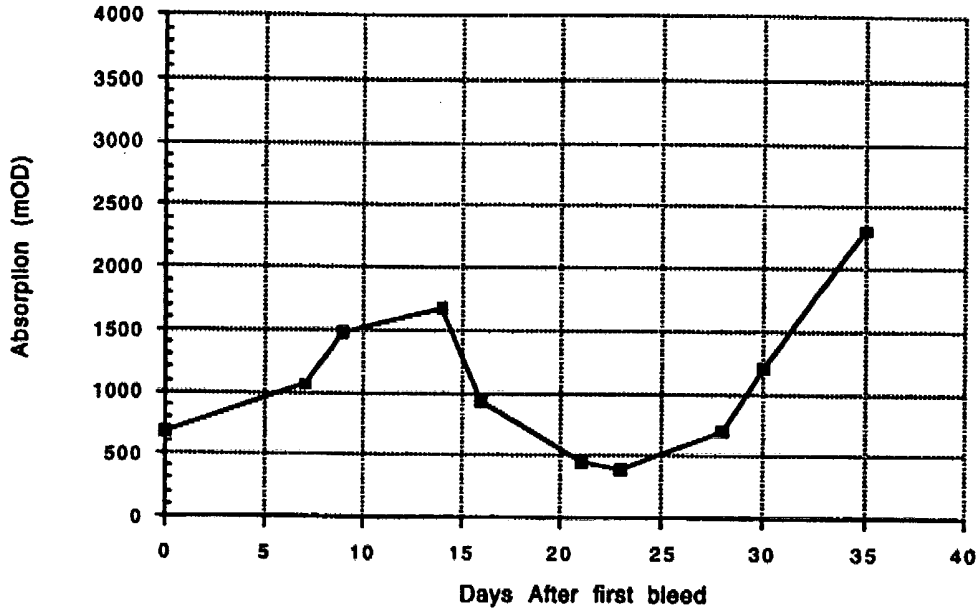
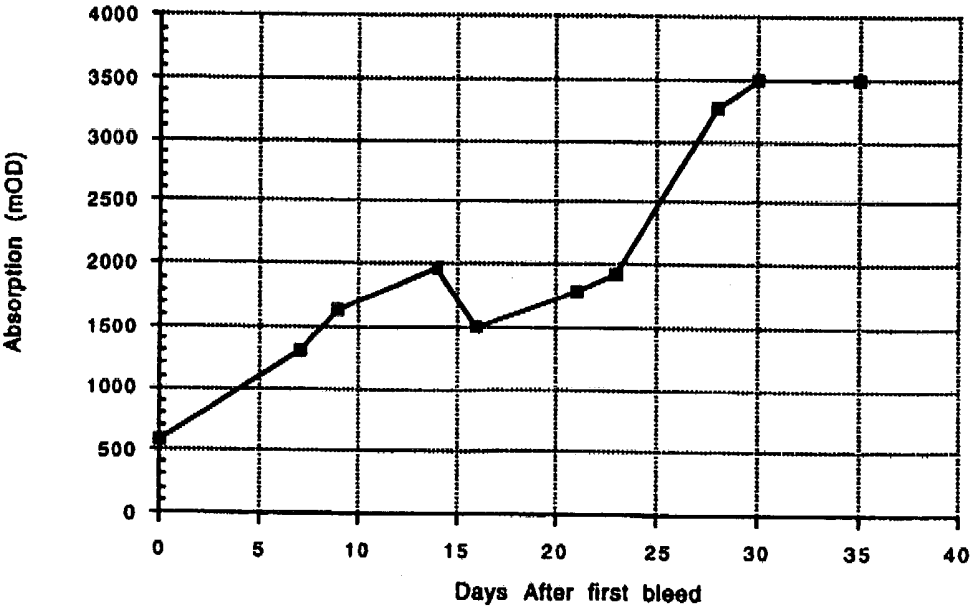


Figure 6



The present invention relates to the simultaneous detection of antigens and antibodies contained in a sample. More particular, the invention relates to the case where the antigens and the antibodies to be detected are derived from or caused by an human immundeficiency virus (HIV), respectively.

HIV-Double antigen sandwich assays (DAGS), which have been developed for the detection of antibodies raised against an antigen, show an initial rise of signal during seroconversion. However, the signal drops in some seroconversion panels again some time later in seroconversion and finally increases again.

This effect is based on the current test structure and on the behavior of the immunglobulins (antibodies) raised by an individual against the antigens of an antigenic organism and can be explained in case of an HIV infection as follows:

1.) The initial rise is mainly caused by anti-envelope (env) HIV-1 low affinity IgM antibodies raised against this antigen. After class switch from IgM to IgG the newly formed anti-env IgG are initially not or poorly detected by an DAGS assay because
a) the density of antigen in the test kit necessary for detecting IgM is such high, that both variable IgG arms are bound to the antigen and no arm remains free to be detected by the added specific antigen conjugate or

b) the affinity of the early formed IgG is too low to remain bound with only one arm bound to the antigen. As a result the signal drops if a sample is taken at the time this class switch has occured and lower amount of IgM and mainly low affinity IgG is present.

2) However, the density of antigen on the solid support as capture reagent must be very high, to be able to bind low affinity IgM, which preferentially binds by avidity. This means the average space in beetween single antigen molecules on the solid support has to be such, that more than one variable region of the antibody can bind to the antigen. Avidity, the binding of an antibody with more than one binding site, is essential for

detection of IgM because the low affinity of a single variable IgM binding site is not sufficient to bind IgM to the solid support by a single binding event.

3) Consequently, high antigen coating density makes it hard to detect antibodies other than class IgM (10 binding sites) or IgA (4 binding sites) in the DAGS test format. IgG, IgE or IgD only possess two binding sites. In case of high antigen density on the solid support both binding sites would be occupied. In the DAGS test format one binding site of the antibody is required for binding via an antibody capture reagent, e.g. an antigen, to a solid support. The second binding site is required for binding the detection reagent. If both binding sites are involved in binding to the solid support the detection reagent can not be bound and the binding of these antibodies remains silent and the signal drops, if these antibodies become the dominant type for a given antigen. In contrast some of the 10 binding sites of IgM are very likely not to be involved in binding to the solid support and therefore IgM is well detected.

The intrinsic association constant that characterizes the binding of an antibody with an epitope or hapten is termed affinity. When the antigen consists of many repeating identical epitopes or when antigens are multivalent, the association between the entire antigen molecule and antibodies depends not only on affinity between each epitope and its corresponding antibody but also on the sum of the affinities of all epitopes involved. While the term affinity denotes the intrinsic association constant between antibody and an univalent ligand such as a hapten, the term avidity is used to denote the overall binding energy between antibodies and the multivalent antigen. Thus, in general, IgM antibodies have higher avidity than IgG antibodies, although the binding of each Fab in the IgM antibody with ligand may be of the same or lower affinity as that of the Fab from IgG. This phenomenon is described on page 105 in "Immunoassys, A short course. 2nd Ed., 1991 Benjamin E. and Leskowitz S.; Liss, A John Wiley & Sons, Inc., Publication.

In practice the separation between IgM and IgG detection is not as unambiguous as described above. The IgM detection system can serve also for detecting IgG, if the concentration of high affinity IgG against the antigen is high enough later after seroconversion (see also Fig.2). However, a second diagnostic window appears as described below. Seroconversion means that the serum or plasma of a previously found negative patient is found positive (reactive) in a later bleed. Typically, the term "seroconversion"

is used for the periode when early markers of infection become measurable. In case of HIV this means the emerging of HIV specific nucleic acids, proteins or early antibodies. In HIV assays a first diagnostic window exists starting from the time of infection with the virus until the earliest possible detection of HIV antigens and of antibodies raised against these antigens. Moreover, a second diagnostic window may exists which is characterised by lower antibody signals during the period when subtypes switch from IgM to IgG antibodies for a certain antigen takes place and these antibodies are not equally well detected by a double antigen assay.

To overcome the problem of the second diagnostic window at least two different antigens or epitopes are used in DAGS format assays. One antigen preferentially for the binding of early IgM and another antigen for the binding of IgG. The IgG detecting antigen is characterized by its ability to compensate or overcompensate the drop of signal after initial rise of the IgM signal for the first antigen. In case of HIV antibody detection assays the IgM detecting antigen usually is part of the env region, the IgG detection antigen is usually from the gag HIV region. An assay for the detection of two antibodies against gp41 and p24 of HIV in a double antigen sandwich format is described in European patent application Publ. No. 386 713.

As an alternative, assays for the simultaneous detection of HIV antigens and HIV antibodies in a sample have been described in the International application Publ. No. WO 93/21346 and in European patent application Publ. No. 0 595 211 A1. The additional detection of an antigen beside detecting antibodies raised against another antigen may allow detecting the presence of an HIV in a sample in a earlier stage of infection, than with an assay detecting antibodies only thereby closing the first diagnostic window slightly.

However, in these applications only the detection of one antigen and of antibodies raised against one single other, different antigen of an HIV has been described. In WO 93/21346 HIV-1 infection is detected via detecting the antibodies which have been raised against the HIV-1 gp41 protein and by detecting the HIV-1 p24-antigen via a pair of p24 antibodies. Addition of the corresponding HIV-2 gp41 protein allows the detection of antibodies for an HIV of another distinct group (HIV-2) in the same assay. Moreover, EP 595 211 describes also the use of an anti HIV p24 antibody in combination with up to three additional env antigens (env gp120 of HIV-1, env gp41 of HIV-1

and env gp32 (36) of HIV-2) derived from two different HIV groups to achieve the detection of at least one of them when present in the sample.

These assays, which only detect antibodies against env antigens of an HIV which belongs to a different HIV group, have an inborn insufficency which could cause false negative results. False negative results could occure in particular after seroconversion, meaning that the described assays would in some seroconversion panels first indicate reactive results above cut off, later indicate results below cut off (not reactive) and eventually give results above cut off (N. Burin des Roziers et al. AIDS No.5 (1995) 528-529). Such a behaviour is not acceptable in viral or any other kind of diagnostics. Such a drop is shown e.g., in WO 93/21346 on page 24. In panel SV0071 sample 60 drops below cut off in case of an antibody/antigen combination assay based on gp41/p24. The p24 antigen itself may also no longer be present in the sample because it is complexed with anti p24 antibodys. In such assays both the presence of an antigen as well as antibodies raised against another antigen of an HIV might not be detected without having a drop in the overall signal below cut off due to an unfavorable antibody and antigen status in the seroconversion situation of the sample at the time the sample is taken.

Moreover, the addition of a second env antigen (V3 loop of gp120) in the assay to detect a second env antibody analyte as described in EP 595 211 does not help in this regard because this loop is not as antigenic in seroconversions as p24 to close the second diagnostic window. Additionally, HIV-1 subtypes are not detected with this variable domain.

Brief Description of the Figures

Figures 1-6 summarize the different absorbances meassured or expected for the same seroconversion panel obtained from an HIV-1 infected person if different known test formats for detecting antibodies and/or antigens and the format of the present invention are applied. Generally a sandwich type assay for detecting an antigen and for detecting an antibody has been used (Fig. 1, Fig. 2, Fig. 4) or is presumed (Fig. 3, Fig. 5, Fig. 6).

Fig. 1. Detection of HIV antibodies using the corresponding p24 antigen. The signal steadily increases due to the increasing amount of antibodies formed.

Fig. 2 Detection of antibodies against the HIV gp41 protein. gp41 is coated at high concentration onto the solid support, yielding a high antigen density. Anti-gp41 IgM are initially bound, resulting in an increase of the measureable signal. After classwitch from IgM to IgG the detectable signal decreases. This is a) due to lower concentration of anti-gp41 IgM antibodies and b) due to either quenching of the anti-env IgG signal because both binding sites are bound to the solid phase or low affinity IgG are no longer bound after the 1. wash step. During progression of the immuneresponse the anti-env-IgG further mature to high affinity antibodies and their concentration increases as well, eventually resulting in a high measurable signal.

Fig. 3. The detection system for the p7 (NC) antigen is shown and the results which can be expexted when a pair of corresponding antibodies raised against it will be used. The meassurable concentration of the antigen will slightly decrease due to the increasing amount of antibodies raised against the antigen in the infected person. For p7 this effect is significantly lower than for gag p24. (see Judith N. Peisen, Master Thesis, 1991, Grad. School of Hood College, USA)

Fig. 4. The result for the combined detection of antibodies against two antigens (gp41 and p24) is shown. This corresponds to the case when the antibody detection systems described in Fig 1 and Fig. 2 are combined. The gag detection system closes the second diagnostic window left open by the env detection system during day 16-30. This system corresponds to the one exemplified in EP 386 713.

Fig. 5. Shows the result which can be expected if the detection systems described in Fig. 2 and Fig. 3 would be combined. The assay will be able to detect an earlier bleed than the system described in Fig. 4. However, the second diagnostic window remains open.

Fig. 6. The assay of the present invention combines the detection systems of Fig. 1, 2 and 3. This assay will detect the same early bleed as the assay described in Fig. 5 but with the gag-detection system (Fig.1) it will also close the second diagnostic window described in Fig. 5 and Fig. 2.

As outlined above, the problem to be solved is to avoid the second diagnostic window in antigen/antibody detection. The present invention provides a solution for this problem with an assay to simultaneously detect

the presence of antigen and antibody analytes of an HIV in a sample, the assay comprises a) detecting at least one HIV antigen analyte in said sample and b) detecting at least two antibody analytes in said sample specific for at least two other HIV antigens of the same HIV, wherein one antibody analyte binds to an epitope which is specific for an HIV envelope antigen and a second antibody analyte binds to an epitope which is specific for an HIV gag antigen, wherein the antigen analytes in step a) are different from the antigens of step b). In particular, the HIV gag antigen is the p24 (capsid, CA) protein in case of HIV-1 or it is a CA protein corresponding thereto occuring in other HIV types. The p24 protein is one protein out of several proteins from the gag region of HIV. Unless otherwise specifically mentioned p24 and proteins corresponding thereto are summarized by the expression "capsid antigen".

In other words the diagnosis of the presence of an HIV in a sample consists of at least three steps: 1.) detecting at least one antigen analyte of said HIV directly, 2.) detecting at least antibodies raised against an env antigen of said HIV, and 3.) detecting antibodies raised against the capsid antigen of said HIV.

Moreover, any antigen analyte can be detected other than the antigens against which antibodies have been raised and which antibodies are selected to be detected as analytes by the present assay.

The drop seen when using only one antigen is compensated by detecting antibodies raised not only against one antigen but also against at least a second antigen. This eliminates the potential second diagnostic window in the known assays detecting one specific antibody and one specifc antigen. Moreover, the present assay configuration provides a possibillity for specifically detecting the presence of an HIV in a sample in a very early stage of the infection by detecting envelope antibody analytes and gag antibody analytes. Since antibodies against the corresponding antigens are raised both very early it is possible to use all type of antibodies (e.g. IgM, IgG) raised against the env and capsid antigens of the HIV and the HIV gag antigens other than the capsid beeing present in a sample.

More specifically the assay of the present invention comprises the steps of simultaneously or sequentially contacting the sample with

a) at least one antigen capture reagent which is an antibody or a fragment thereof specific for an epitope in a gag antigen analyte,

b) at least two antibody capture reagents wherein
one antibody capture reagent comprises an epitope which is specific for an envelope antibody analyte, and
one antibody capture reagent comprises an epitope which is specific for a capsid antibody analyte, and
c) detecting the antigen/antigen capture reagent and antibody/antibody capture reagent complexes,
provided that the gag antigen analyte is not the capsid protein.

The term epitope or antigenic determinant as used in the present specification is any subsequence within a naturally occuring HIV protein which is recognised by the immune systeme and causes the production of antibodies against this protein. These epitopes provide the specificity based on which antibodies bind to a specific protein only. Moreover, a compound (e.g. a protein or polypeptide) is considered as an "antigen" when it contains at least one epitope.

Therefore, the whole protein but also fragments of this protein, which contains an epitope, can be used for the production of antibodies against these epitopes, which then in turn can be used as an antigen capture reagent. Alternatively, they can be used for the detection of antibodies already raised against this epitope by making use of this protein or antigenic fragments thereof as an antibody capture reagent. In all these cases the capture reagents are derived from a particular protein having suitable epitopes existing within.

More than one antigen analyte of an HIV may be detected. The antibody analytes are also not limited to an antibody analyte specific for an env and a capsid antigen. Several distinct HIV gag and env proteins do exist. For example, although an env antibody analyte specific for an epitope in the envelope gp41 protein of HIV-1 is detected, another antibody analyte specific for an epitope in the envelope gp160 protein of HIV-1 may also be detected since gp41 is a part of this precursor molecule. The same applies to the env proteins of other types and subtypes of HIV's.

Alternatively, also an antibody analyte specific for a polymerase or integrase antigen may be detected in addition to the env and gag antibody analyts. The same antigens may be used as antigen capture reagents when antibodies raised against these antigens are prepared.

In a preferred embodiment of the assay for each HIV one gag antigen analyte is detected and one antibody analyte for an envelope antigen and one antibody analyte for the capsid antigen are detected.

Any HIV infection may be detected with the assay. The antigen analytes and the antigens which are detected via their antibody analytes belong to the same HIV. Accordingly, the antigen analytes and the antibody analytes belong to a virus from the group of HIV-1, HIV-2 or to a virus from the group of HIV-3.

This also applies to any distinct subgroup within each of the three major groups. For example HIV-1 is devided into several distinct subgroups such as A-H and O as described in Myers G, et al.: Human Retroviruses and AIDS. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, New Mexico, 1993; Meyers G, Korber B., Wain-Hobson S., Smith R.F., and Pavlakis G.N. in Human Retroviruses and AIDS. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, New Mexico, 1993; Gürtler et al. in J. Virol. 68 (1994) 1581-1585; Cheingsong-Popov R. et al. in AIDS Research and Human Retroviruses 10 (1994) 1379-1386. The nomenclature for the third group (HIV-3) is currently not quite clear. However, viruses belonging to this group are distinct and, therefore, may also be regarded as a subgroup of HIV-1 or HIV-2. For a discussion of HIV-1, HIV-2 and HIV-3 see below.

In another embodiment not only one type of HIV but two or more types of HIV's may also be detected simultaneously by this assay. On the one hand, for each type of virus to be detected an individual set of capture reagents for the corresponding antigen and antibody analytes can be used. This means that in the assay of the present invention additional antigen and/or antibody capture reagents specific for a gag antigen or envelope antibody analyte of another HIV are added.

On the other hand, it is known that, although two HIV belong to different groups such as HIV-1 and HIV-2, or one of the two HIV belongs to a subgroup, a particular antigen or antibody analyte of one HIV is also crossreactive with one or more antigen or antibody analytes of another HIV. In this embodiment of the assay of the present invention the detection of a variety of HIV's, if present, either alone or combined, in the sample is possible. Accordingly, by choosing the appropriate capture reagents, an HIV belonging to a specifc group or subgroup may only be detected or any HIV

present in the sample which crossreacts with the capture reagents may be detected by the given set of capture reagents. Preferably, the capsid antigens are suitable in this regard for detecting corresponding antibody analytes. In this embodiment, the capture reagent for the capsid antibody analyte crossreacts with an antibody analyte for the capsid antigen of another HIV.

The detection of envelope and gag antibody analytes has already been mentioned above. The assay of the present invention advantageously makes use of the circumstance that different classes of antibodies specific for these two groups of antigens occur at overlaping times which can be detected separately.

In a preferred embodiment of the assay for detecting the envelope and gag antibody analytes the analytes to be detected are such that the antibody analyte specific for the envelope antigen is a low affinity antibody, and the antibody analyte specific for the capsid antigen is a high affinity antibody.

Most preferred, the antibodies detected in the assay of the present invention are such that the low affinity antibody is IgM and the high affinity antibody is IgG, IgA and/or IgE.

A low affinity antibody binds by way of avidity whereas a high affinity antibody binds by way of its affinity as described above in order to be detected by the assay. In detecting different classes of immunglobulins, the assay has the advantage to be more sensitive than assays which detect IgG only.

However, it is understood that also antibody analytes specific for a third or more antigen can be detected with the assay of the present invention. In this case, the antibodies to be detected can be low and/or high affinity antibodies.

The detection of the antigen and antibody analytes according to the basic principles outlined above may be performed in various way. The analytes may be detected in a heterogenous assay, which means that the analytes (antigens, antibodies) are bound to a surface and separated from the sample before detection. However, the assay of the present invention can also be performed in a manner wherein the presence of the antigen analytes and the antibody analytes is determined in solution. The term solution assay comprises an assay wherein the antigens and antibodies are detected by interaction with their respective specific indicator reagents in solution.

Moreover, in case the indicator consists of some solid supports the assay may also be performed as a heterogeneous separation free assay. For example, if 20-500 nm in diameter particles are used as the solid phase in a particle-enhanced turbidumetric immunoassay (R.M. Nakamura & Rippey, J.H. (eds), Diagnostic Immunology: Technology Assessment, College of American Pathologists, Skokie IL, 1983, pp. 18-30), as particle-enhanced light scattering (nephelometric) immunoassay (Henneberry, H & Price, C.P., Ann. Clin. Biochem. 29, 1992, 22-42) or as particle agglutination assay (in: R.F. Masseyeff, W.H. Albert &N.A. Staines (eds), Methods of immunological Analysis, VCH Verlag 1993, pp.203-214) or in combination with other feasible detection methods. In such type of assays the indicator reagent alone, in combination with the analyte and the support, are sufficient to detect the analytes in the sample.

In a preferred embodiment the assay of the present invention is preformed as a heterogeneous assay, which means that the analytes are attached or bound to a support and separated from the sample before they are detected. Most preferred the assay is performed in a manner wherein the antigen analytes and the antibody analytes are detected by a sandwich immunoassay.

This means that the antigen analytes and the antibody analytes are detected by contacting the analytes with
a) at least one antigen indicator reagent which is an antibody or a fragment thereof specific for an epitope in the gag antigen analyte, and
b) with at least two antibody indicator reagents wherein
one antibody indicator reagent comprises an epitope which is specific for the envelope antibody analyte, and
one antibody indicator reagent comprises an epitope which is specific for the capsid antibody analyte,
and wherein each of the antibody indicator reagents has the same epitope specificity as the corresponding antibody capture reagent.

Since only the same epitope specificity is required, the capture reagent and the corresponding indicator may differ completely in their overall structure. For example, the capture reagent may be the whole HIV protein whereas the indicator reagent is only a peptide or vice versa.

The antigen indicator reagent is an antibody specific for an epitope in that antigen analyte. Preferably, the epitope is different from the epitope

recognized by the antigen capture reagent. However, in case the antigen consists of dimers or oligomers, the epitopes recognised by the capture and the indicator reagent may be the same.

In the practice of the present invention, the manner in which the assay is performed is not critical and, therefore, the assay can be performed in various different ways. In one embodiment to detect the presence of at least one HIV in a sample by detecting
at least one HIV antigen analyte in said sample and
at least two antibody analytes in said sample specific for at least two other HIV antigens of the same HIV, wherein one antibody analyte binds to an epitope which is specific for an HIV envelope antigen and a second antibody analyte binds to an epitope which is specific for an HIV gag antigen,

the assay is characterized by

i) simultaneously or sequentially contacting the sample with:

a) at least one antigen capture reagent specific for an epitope in a gag antigen analyte which reagent is or can be attached to a solid support;

b) at least two antibody capture reagents,
wherein one antibody capture reagent comprises an epitope which is specific for an envelope antibody analyte
and one antibody capture reagent comprises an epitope which is specific for a capsid antibody analyte, and
said antibody capture reagents are or can be attached to a solid support;

c) at least one antigen indicator reagent specific for the gag antigen analyte,
the indicator reagent is labelled or can be detected by a secondary label,

d) at least two antibody indicator reagents wherein
one antibody indicator reagent comprises an epitope specific for the envelope antibody analyte and
at least one antibody indicator reagent comprises an epitope specific for the capsid antibody analyte,
which indicator reagents are labelled or can be detected by a secondary label;

thereby forming
antigen capture reagent/gag antigen/antigen indicator reagent complexes,

antibody capture reagent/envelope antibody/antibody indicator reagent complexes and
antibody capture reagent/capsid antibody/antibody indicator reagent complexes, and

ii)(a) binding of the complexes to a solid support, if the capture reagents are not already bound to a solid support,

b) separating the solid support from the liquid phase,

c) optionally adding a secondary label before or after step b) to the mixture which binds to the antibody and/or antigen indicators, if one or more of the indicator reagents do not already contain a label, and

iii) determining the presence of the antigen analytes and the antibody analytes in the sample by detecting the corresponding signal generated by the labels.

In a second embodiment the assay is characterized by
i) simultaneously or sequentially contacting the sample with:

a) at least one antigen capture reagent specific for an epitope in a gag antigen analyte which reagent is or can be attached to a solid support;

b) at least two antibody capture reagents,
wherein one antibody capture reagent comprises an epitope which is specific for an envelope antibody analyte
and one antibody capture reagent comprises an epitope which is specific for a capsid antibody analyte,
said antibody capture reagents are or can be attached to a solid support; and

c) at least one antigen indicator reagent specific for the gag antigen to be detected,
the indicator reagent is labelled or can be detected by a secondary label;

thereby forming a first mixture of
antigen capture reagent/gag antigen/antigen indicator reagent complexes,
antibody capture reagent/envelope antibody complexes and
antibody capture reagent/capsid antibody complexes, and

ii) a) binding of the complexes to a solid support, if the capture reagents are not already bound to a support,

b) separating the solid support from the liquid phase,

c) optionally adding a secondary label to the mixture before or after step b) which binds to the antigen indicator reagent, if the antigen indicator reagent does not already contain a label, and

iii) a) contacting said complexes on the solid support with at least two antibody indicator reagents, wherein
one antibody indicator reagent comprises an epitope specific for the envelope antibody analyte and
one antibody indicator reagent comprises an epitope specific for the capsid antibody analyte,
the indicator reagents are labelled or can be detected by a secondary label, thereby forming a second mixture,

b) adding a secondary label to the mixture which binds to the antibody indicator reagent, if one of the antibody indicator reagents does not already contain a label, and

iiii) determining the presence of the antigen analytes and the antibody analytes in the sample by detecting the corresponding signal generated by the label.

In a third embodiment the assay is characterized by
i) simultaneously or sequentially contacting the sample with

a) at least one antigen capture reagent specific for an epitope in a gag antigen analyte which reagent is or can be attached to a solid support,

b) at least two different antibody capture reagents,
wherein one antibody capture reagent comprises an epitope which is specific for an envelope antibody analyte
and at least one capture reagent comprises an epitope which is specific for a capsid antibody analyte,
said antibody capture reagents are or can be attached to a solid support,

thereby forming a first mixture of
antigen capture reagent/gag antigen complexes,
antibody capture reagent/envelope antibody complexes and
antibody capture reagent/capsid antibody complexes,

c) binding of the complexes to a solid support, if the capture reagents are not already bound to a support, and

b) separating the solid support from the liquid phase, and

ii) a) contacting said complexes on the solid support with at least one gag antigen indicator reagent and at least two antibody indicator reagents wherein one antibody indicator reagent comprises an epitope specific for the envelope antibody analyte and at least one antibody indicator reagent comprises an epitope specific for the capsid antibody analyte which indicator reagents are labelled or can be detected by a secondary label,

thereby forming a second mixture, and

b) adding a secondary label to the mixture which binds to the antigen and/or antibody indicator reagents, if one of the indicator reagents does not already contain a label, and

iiii) determining the presence of the antigen analytes and the antibody analytes in the sample by detecting the signal generated by the label.

The assay of the present invention may also be performed in all ways apparent for those skilled in the art by varying the incubation steps. For example, in an assay consisting of only one wash step before adding of the indicator substrate (one-step assay) all antibody or antigen indicator reagents (indicator reagents) are added during the first incubation step. In case of a second wash cycle the indicator reagents can all be added in the second step. In case there is more than one indicator reagent, the addition of these different indicators can be split up amongst first and second incubation step (see table below). Depending on the individual desires of a certain assay all permutations can be tested and the best one selected. In case there are more than 2 incubation steps (n-steps) the different indicator reagents can be split up in any combination amongst all n incubation steps. In the more defined situation, with two incubation steps and 3 indicator reagents (A = antigen indicator, B = antibody 1 indicator, C = antibody 2 indicator) it is possible to add the indicators according to the following table:

Step 1	Step 2

A + B + C	—
A + B	C
A + C	B
B + C	A
A	B + C
B	A + C
C	A + B
—	A + B + C

In the above table the first situation corresponds to a one step format and the last one to a two step format, respectively. The others belong to a one step/two step assay format. The description in this table refers to the situation where one type of HIV should be detected. (e.g. HIV-1 only). In case the assay has to be adapted to detect also another HIV (e.g. variants of HIV-1 or HIV-2, or HIV-3 or yet to discover variants) the according additional indicators, if necessary, can be added to any possible combination mentioned above. Accordingly, the assay of the present invention is able to detect the presence of an HIV in the sample wherein the antigen analytes and the antigens detected via their antibody analytes belong to a virus from the group of HIV-1, HIV-2 and/or HIV-3.

Positive and negative controls can be included in the assay of the present invention to ensure reliable results. Positive controls can include a positive control for each analyte which control is tested separately, and a combined positive control wherein the presence of all analytes to be detected in the assay are determined. Negative samples can be included as negative control.

The sample can be a mammalian biological fluid such as whole blood or whole blood components including red blood cells, white blood cells including lymphocyte or lymphocyte subset preparations, platelets, serum, plasma or ascites, saliva, stools, cerebrospinal fluid, urine, sputum, tracheal aspirates and other constituents of the body which may contain or

be suspected of containing the antigen and antibody analytes of interest. The test sample also can be a culture fluid supernatant, or a suspension of cultured cells. Mammals whose body fluids can be assayed for antigen and antibody analyte according to the present invention include humans and primates, as well as other mammals who are suspected of containing these antigen and antibody analytes of interest. Human plasma and serum are preferred.

The capture reagents of the present invention are specific for each of the antigen analyte or antibody analyte of interest. However, as mentioned above one or more capture reagent can be used in the assay of the present invention wherein the capture reagent specific for the antigen or the antibody analyte of one HIV is also crossreactive with an antigen or antibody analyte of another HIV.

Moreover, in performing the assay of the present invention the antigen capture reagent and the antibody capture reagents are bound or can be bound to a solid support.

According to the antigen and the antibodies to be detected, the capture reagent as used in the present invention is an immunoreactive compound such as an antibody, antigen, or antibody/antigen complex. The antibody used as a capture reagent for the antigen analyte can be a monoclonal antibody, a polyclonal antibody, an antibody fragment, a recombinant antibody, a mixture thereof, or a mixture of an antibody and other specific binding members which are specific for an epitope in that antigen analyte. Methods for producing antibodies against an antigen are known in the art and are in general described by Köhler and Milstein in Nature 256 (1975) 495-497.

The antibody capture reagent comprises an epitope which is specific for the antibody analyte. Usually, the capture reagent is a polymer of amino acids (polypeptide). This polypeptide can be a protein, protein fragment or a peptide which is recombinantly or synthetically produced. Alternatively, it may be obtained from natural sources, e.g. from cell cultures and the like. It may be used as whole protein or beeing a fragment thereof including peptides larger than 6 amino acids. Preferably, such a peptide is between 10 to 50 amino acids in length. Also a fusion polypeptide comprising the epitope which is necessary for antibody capture and having additional amino acids not contained in the relevant protein of the HIV may be used. Furthermore,

instead of using two or more separate antibody capture reagents, only one fusion polypeptide may be used which comprises two or more different binding members (epitopes) each of which are specific for the corresponding antibody analyte. In such a case, a fusion protein with epitopes specific for two antibody analytes is preferred

Of course, the two or more antibody capture reagents used in the present invention are directed against antibodies with different antigenic or epitopic specificity.

The capture reagent can be directly or indirectly bound (attached) to a solid support before or during the performance of the assay, thereby enabling the separation of immobilized complexes from the test sample. This attachment can be achieved directly, e.g., by coating the specific binding member onto the solid support, by absorption or covalent coupling. The terms "bound" and "attached" are used interchangeable in the present description.

Alternatively, for the indirect capture of the capture reagent to a support, the solid support can retain an additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. The receptor can also be any specific binding member which is immobilized or attached to the solid support and which has the ability to immobilize the capture reagent through a specific binding reaction. The receptor molecule enables the indirect binding of the capture reagent for the antigen or the antibody analyte to a solid support material before the performance of the assay or during the performance of the assay. Coating methods, and other known means of attachment, are known to those in the art.

A preferred coupling method for the assay of the present invention is the indirect attachment of the capture reagent via an additional rezeptor. Such a system may consist of any fluoresceine derivative and its corresponding antibody thereto as in the Fluoresceinisothiocyanat/anti-Fluoresceinisothiocyanat antibody (FITC/antiFITC) system. Also the Biotin/ Streptavidin, Biotin/Avidin or Biotin/anti-Biotin antibody system may be used for the indirect capture of the antigen or antibody capture reagent. Other ligand/receptor system known to those skilled in the art can be used as well.

Another posibility are ion capture procedures for immobilizing an immobilizable reaction complex with a negatively charged polymer as described in EP Publication Nos. 326 100 A2 and 406 473 A1. They can be employed in an assay of the present invention to effect a fast solution-phase immunochemical reaction. Such an immobilizable immune complex is separated from the rest of the reaction mixture by ionic interactions between the negatively charged polyanion/immune complex and the previously treated, positively charged porous matrix and detected by using various signal generating systems as described below.

The capture reagents can be attached to the same solid support, or can be attached to different solid supports. It is contemplated that all capture reagents can be attached to the same solid support, or that each capture reagent can be attached to a separate solid support. This means that the antigen capture reagent and the antibody capture reagents are bound or can be bound to the same support or to different supports. In a preferred embodiment, a combination of capture reagents can be attached to separate solid supports. For example,

a) one antigen capture reagent is bound or can be bound to a first support and

b) the two antibody capture reagents specific for the envelope and the capsid antibody analytes are bound or can be bound independently either alone or in any combination to the first and/or to a second support or to a second and/or a third support.

In case different HIV 's are detected, the HIV-1 antigen and antibody capture reagents specific for one HIV may be bound to one support and one or more capture reagents specific for those of the other HIV may be bound to a second support. Accordingly, one antigen capture reagent and two antibody capture reagents as defined herein which are specific for HIV-1 can be bound to one support and one capture reagent specific for an HIV-2 env antibody can be bound to a second support. However, depending on the number (n) of antigens and antibodies to be detected up to n supports may be used separately.

If microparticles were the solid support of choice, then separate microparticles can have at least one capture reagent attached to it. A mixture of microparticles (solid supports) can be used to capture the various antigen and antibody analytes which may be present in the test sample by using the mixture of microparticles.

It is contemplated that different ratios of capture reagents attached to a solid support can be utilized in such an assay, to optimize antigen and antibody detection especially for detecting the aforsaid low and high affinity antibodies. In order to detect low affinity IgM it is preferred to use a high density of antigen. The average distance of the antigen molecules has to be such that an IgM can bind with at least two variable regions. This type of binding is referred to as avidity. Binding with one variable region only would not be sufficient for permanent immobilization.

In case the density of the capture reagent for the IgG analyte is extremly high, the average reagent density might become such that the IgG bind with both variable regions. As a result the specific indicator reagent can no longer bind to the high affinity antibody. This is in particular a problem if only low concentration of IgG is present. In such a case the assay may be modified such that the antigen density is adjusted accordingly. The average distance of the antigen molecule should be such that an IgG binds only with one variable region. This type of binding is referred to as affinity. Due to the high affinity of the antibody, one binding event is sufficent for permanent immobilization.

Therefore, in order to detect high affinity IgG or other high affinity immunoglobulins in the DAGS test format a lower density of antigen is preferred. A low density means that an IgG signal is not quenched. The relative density can be indirectly determined by comparing samples known for having either a high IgM or a high IgG level for a certain HIV antigen. Depending on which sample yields a higher value the corresponding support can be classified as having a relatively lower or higher density of a certain antibody capture reagent which is bound or can be bound to the support.

In a particular embodiment of the assay of the present invention the antibody capture reagents are employed such that

a) the antibody capture reagent specific for the envelope antibody analyte is bound to a support or can be bound to a support in a density for binding and detecting a low affinity antibody, and

b) the antibody capture reagent for the capsid antibody analyte is bound to a support or can be bound to a support in a density for binding and detecting a high affinity antibody.

Such methods for regulating the density of the capture reagents, either antigens or antibodies, attached directly or indirectly to a support, are known to a person skilled in the art and are controled by the coating conditions of the solid phase. In particular, variation of antibody capture reagent concentration, antibody capture reagent modification of any kind, pH, salt concentration and selection of detergent and concentration of detergent determine the final concentration of the antibody capture reagent bound on the solid phase.

The solid support is not critical and can be selected by one skilled in the art. It refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid support can be a plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface of a test tube, microtiter well, sheet, bead, microparticle, chip, membrane, and other configurations known to those of ordinary skill in the art.

It is contemplated that all solid supports be present during the quantitation of signal if a heterogenous assay is performed, thus eliminating the need to separate solid supports for detecting the signal. The solid support can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Suitable methods for immobilizing capture reagents on solid supports include ionic, hydrophobic, covalent interactions and the like. Methods for attaching the capture reagents are also described above under the capture reagent.

The solid support also can comprise any suitable porous material with sufficient porosity to allow access by detection antibodies and a suitable surface affinity to bind antigens. Microporous structures are generaily preferred, but materials with gel structure in the hydrated state may be used as well. Other useful solid supports include: Natural polymeric carbohydrates and their synthetically modified, cross-linked or substituted derivatives; natural polymers containing nitrogen, such as proteins and derivatives, including crosslinked or modified gelatins; natural hydrocarbon polymers, such as latex and rubber;
synthetic polymers which may be prepared with suitably porous structures, such as vinyl polymers, polyacrylamides, polymethacrylates, copolymers and terpolymers of the above polycondensates, such as polyesters;
porous inorganic materials such as sulfates or carbonates of alkaline earth metals and aluminum or silicon oxides or hydrates.

All of these materials may be used in suitable shapes, such as films, sheets, beads or plates or they may be coated onto or bonded or laminated to appropriate inert carriers, such as paper, glass, plastic films, or fabrics. The porous structure of nitrocellulose has excellent absorption and adsorption qualities for a wide variety of reagents including monoclonal antibodies. Nylon also possesses similar characteristics and is suitable. It is contemplated that such porous solid supports described hereinabove are preferably in the form of sheets. The pore size may vary within wide limits, and is preferably from about 0.025 to 15 microns, especially from about 0.15 to 15 microns. The surfaces of such supports may be activated by chemical processes which cause covalent linkage of the antigen or antibody to the support. The irreversible binding of the antigen or antibody is obtained, however, in general, by adsorption on the porous material by hydrophobic forces.

Preferred solid support materials for flow-through assay devices include filter paper such as a porous fiberglass material or other fiber matrix materials. The thickness of such material is not critical and will be a matter of choice, largely based upon the properties of the sample or analyte being assayed, such as the fluidity of the test sample.

To change or enhance the intrinsic charge of the solid support, a charged substance can be coated directly to the material or onto microparticles which then are retained by a solid support material. Alternatively, microparticles can serve as the solid support, by being retained in a column or being suspended in the mixture of soluble reagents and test sample, or the particles themselves can be retained and immobilized by a solid support support material. By retained and immobilized is meant that the particles on or in the support material are not capable of substantial movement to positions elsewhere within the support material. The particles can be selected by one skilled in the art from any suitable type of particulate material and include those composed of polystyrene, polymethylacrylate, polypropylene, latex, polytetrafluoroethylene, polyacrylonitrile, polycarbonate, or similar material.

Also, the methods of the present invention can be adapted for use in systems which utilize microparticle technology including automated and semiautomated systems wherein the solid support comprises a microparticle. Such systems include those devices as described e.g. in EP

patent application publication No. EP 0 424 634 A2 or in U.S. Patent No. 5,006,309.

The indicator reagent comprises a label conjugated to a specific binding member which binds to each antigen or antibody analyte. Each indicator reagent produces a detectable signal at a level relative to the amount of the antigen and antibody analyte in the test sample. In a preferred embodiment, each indicator reagent, while comprising a specific binding member of a different antigen and antibody analyte, is conjugated to the same signal generating compound (label), which is capable of generating a detectable signal.

According to the antigen and the antibodies to be detected, the binding member in the indicator reagent as used in the present invention is an immunoreactive compound such as an antibody, antigen, or antibody/antigen complex that is capable of binding either to the antigen and antibody analytes as in a sandwich assay. If an antibody is used as an antigen indicator reagent, it can be a monoclonal antibody, a polyclonal antibody, an antibody fragment, a recombinant antibody, a mixture thereof, or a mixture of an antibody and other specific binding members which are specific for an epitope in that antigen analyte.

The antibody indicator reagent comprises an epitope which is specific for the antibody analyte. Usually, the capture reagent is a polymer of amino acids (polypeptide). This polypeptide can be a protein or a peptide which is recombinantly or synthetically produced. Alternatively, they may be obtained from natural sources, e.g. from cell cultures and the like. They may be used as whole protein or beeing a fragment thereof including peptides larger than 6 amino acids. Preferably, the peptide is between 10 to 50 amino acids in length. Also fusion proteins comprising the antigenic region containing a specific epitope necessary for antibody capture and having additional amino acids not contained in the relevant protein of the HIV may be used. Furthermore, instead of using two or more separate antibody indicator reagents, only one fusion protein may be used which comprises two or more different binding members (epitopes) each of which are specific for the corresponding antibody analyte. A fusion protein with epitopes specific for two antibody analytes is preferred.

Usually, the binding member for the antigen analyte is a corresponding antibody. If the capture reagent is also an antibody an antibody sandwich

results. For the antibody analytes, the capture reagent is the corresponding antigen which results in a double antigen sandwich format if also an antigen is used as an indicator reagent.

The signal generating compound (label) of the indicator reagent is capable of generating a measurable signal detectable by external means. The various signal generating compounds (labels) contemplated include chromagens; catalysts such as enzymes for example, horseradish peroxidase, alkaline phosphatase, and betagalactosidase; luminescent compounds such as fluorescein and rhodamine; chemiluminescent compounds such as acridinium compounds (e.g. acridinium ester), phenanthridinium compounds and dioxetane compounds; radioactive elements; and direct visual labels. Chemiluminescent signal measurements using a label are described in EP Publication No. 0 273 115 A2. The selection of a particular label is not critical, but it will be capable of producing a signal either by itself or in conjunction with one or more additional substances. A variety of different indicator reagents can be formed by varying either the label or the specific binding member. Preferably, the label is selected from the group of enzymes, acridinium ester or Ruthenium complexes. Preferably, the enzyme be horseradish peroxidase (HRPO) or alkaline phosphatase.

In the present invention, the total signal generated by the indicator reagent(s) indicates the presence of one or more of the antigen and antibody analytes in the test sample. It is contemplated that different signal generating compounds can be utilized in the practice of the present invention. Thus, for example, different fluorescent compounds could be utilized as the signal generating compounds, one for each indicator reagent, and detection could be determined by reading at different wavelengths. Alternatively, a short-lived chemiluminescent compound such as an acridinium or phenanthridinium compound and a long-lived chemiluminescent compound such as a dioxetane can be utilized to generate signals at different times for different analytes as described in WO 92/12255. Also different enzymes which use different substrates may be used and the corresponding signals generated may be detected. In a particular preferred embodiment the label is the same for all indicator reagents used.

The label can be conjugated to the indicator reagent before the reagent reacts with the antigen or antibody analytes. However, the label may also be conjugated with the indicator reagent after the analyte/indicator reagent

complex has been created. Therefore, the indicator reagent can retain an additional receptor which has the ability to attract and immobilize the label. Such a secondary label can be introduced in the label and in the indicator reagent by any system which allows selective binding of the label to the indicator reagent. Such systems are known to a person skilled in the art. Corresponding systems which have been described above for binding of the capture reagent to a support can be used as well. For example, the use of a biotin/anti-biotin (avidin or anti-biotin antibody) system for immunoassays is described in EP 160 900 A2.

In a preferred embodiment of the present assay, the indicator reagent is already labelled before it is added to the sample.

In a preferred embodiment of the assay the HIV to be detected is HIV-1. HIV-1 and its structure has been described by Ratner et al. (HTLV-III; Nature 313 (1985) 277-284). Variants (other isolates) have been described by Sanchez-Pescador (ARV-2; Science 227 (1985) 484-492) or in WO 86/01827 (LAV). The env protein and other proteins which react as antigens are described in more detail in WO 86/02383.

Env and gag derived HIV antigens, either as whole protein or as a peptide, have been shown for their utility in diagnostic antibody screening assays. However in some cases analytical problems still remain. p24 antigen is only measureable in HIV seroconversions during a couple of days, due to the high immunogenicity of p24. This means that the overall signal can drop below cut off in assays based on one antibody for the p24 antigen detection and one antigen for detecting an antibody raised against a second antigen and the sample appears to be negative since the antibody signal alone may not be sufficient.

Since usually a specific monoclonal or polyclonal p24 antibody has already been used in the known assays described above (WO 93/21346, EP 595 211 A1) for the detection of the p24 antigen, the anti-p24 douple antigen system can no longer be used for the detection of the corresponding p24 antibodies. The use of such a system would result in binding of the p24 conjugate at the p24 antigen detection system and could cause false positive results.

Therefore the p24 antibody system must be ommitted and these assays potentially open a second diagnostic window as already described above. This

is not acceptable in HIV screening assays. However, in case of HIV diagnostic the detection of antibodies other than anti p24 is not feasible, because all HIV antigens other than gp41 and p24 show a lower degree of immunogenicity. Antibodies against other HIV antigens, e.g. gp120, appear later during seroconversion, as can be seen in western blotts.

In accordance with the assay of the invention for HIV-1 the antigen detected preferably is not the commonly used gag p24 (core or capsid, CA) antigen, rather any gag antigen from the HIV-1 gag reading frame except p24. These antigens may be p55 precursor or its fragments p17 (matrix, MA), p2, p7 (nucleocapsid, NC), p1 or p6 from the HIV-1 gag region. Although p24 is part of p55, it is possible to design p55 assays which do not interfere with p24. Therefore, the components are such that the antigen capture reagent is derived from an antigenic region of the p17 (Matrix, MA), p2, p7 (Nucleocapsid, NC), p1, or p6 protein of the HIV-1 gag region. The proteins are referred to according to the description given by Henderson et al. J. Virol 66 1856-65 (1992). Preferably, p17 or p7 or p6 antigen are detected. For other viruses, in particular HIV-2 or distinguishable subtypes the corresponding regions can be used and the same selection applies.

Therefore, a suitable antigen capture reagent is an anti p17, p7 or p6 HIV-1 antibody which is specific for an epitope in that proteins. The preparation of antibodies against the p17 gag protein has been described in published PCT application Publ. No: WO 86/04336. The preparation of antibodies against p7, which can be used in the present invention for detecting the p7 antigen, are described in US Patent No. 5,462,852. The same described procedures may be used for the preparation of antigen capture antibodies in case of the gag proteins of HIV-2 or HIV-3 or any subtypes thereof.

Accordingly, a virus belonging to the group of HIV-1 may be detected in accordance with the assay of the present invention wherein the virus is from the group of HIV-1 and

a) one antigen capture reagent is an antibody or a fragment thereof which is specific for an epitope on the HIV-1 gag proteins p17, p2, p7, p1, or p6,

b) one antibody capture reagent for the envelope antibody analyte comprises an epitope which is specific for the HIV-1 envelope gp160 or gp41 protein, and

one antibody capture reagent for the capsid antibody analyte comprises an epitope which is specific for the HIV-1 core p24 protein.

One of the antibody capture reagents is derived from an antigenic region of the envelope region of HIV-1 (e.g. gp160 or gp41). WO 86/02930 describes the purified gp160.

Preferably, the antibody capture reagent comprises an gp41 antigenic sequence and detects anti gp41 antibodies. Expression of HIV gp41 or parts of HIV gp41 have demonstrated the utility of recombinant DNA derived HIV envelope sequences in diagnostic assays (Chang et al., Biotechnology 3 (1985) 905-909; Crowl et al., Cell 41 (1985) 979-986; Cabradilla et al., Biotechnology 4 (1986) 128-133.

More preferred, the antibody capture reagent specific for the envelope antibody analyte is a peptide or polypeptide comprising the aminoacid sequence CSGKLIC or CSGKIIC from gp41 or a variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form.

The cyclic form is preferred. The sequence CSGKLIC corresponds to amino acids 605-611 of the env region according to the numbering system of Ratner et. al. (Nature 313, 277-284 (1985). This sequence corresponds to a highly conserved epitope in the gp41 protein of various HIV-1 strains.

Corresponding peptides derived from the gp41 protein containing the sequence CSGKLIC, which are suitable as a capture reagent or as a indicator reagent, are described in EP 326 490 A2. In particular the peptides described on page 4 of said application under formula (I) or in claim 1 can be used. As an example for a cyclic S-S peptide for use in the present assay reference is made to peptide 87c or peptide 88 disclosed on page 7, lines 16 and 17 of said European application. However, any other of the disclosed peptides may be used in cyclic or non cyclic form as well.

Variants of this sequence motif in between the two cysteins are, e.g., CSGKHIC, CSGKAVC, CKGKHIC and CKNRLIC, as described in Lancet 345 (1995) 856-857 or CSGKIIC. The sequence CSGKIIC corresponds to subtype E which has been described by McCutchan et al. in AIDS Res. Hum. Retroviruses 8 (11), 1887-1895 (1992)

The capture reagent can be the whole gp41 protein, a part thereof, or a protein containing antigenic sequences thereof. Alternatively, these sequences can be contained in a shorter polypeptide of any length or in a peptide up to about 50 amino acids. Whereas the gp41 antigen preferably be a recombinantly prepared antigen (protein), the polypeptide may be

synthetically or recombinantly prepared. Such a recombinantly prepared env polypeptide from gp41 is described in EP 219 106. EP 201 716 describes also recombinant polypeptides with sequences from gp41.

Moreover, in the assay of the present invention the capture reagent for the low affinity antibody analyte preferably is a reagent which comprises an epitope which is specific for the gp41 as described in the paragraph before and is used for the detection of IgM antibodies.

Next, the second capture reagent for detecting antibodies raised against the gag p24 capsid protein is preferred. The expression of HIV gag proteins in E. coli have indicated that the HIV gag proteins produced by recombinant DNA technology could have potential diagnostic value (Dowbenko et al., PNAS USA 82 (1985) 7748-7752; Steimer et al., Virology 150 (1986) 283-290).

The capture reagent can be the whole p24 protein, a part thereof, or a protein comprising an amino acid sequence corresponding to an epitope specific for the p24 protein. Alternatively, these sequences can be contained in a shorter polypeptide or in a peptide up to 50 amino acids. The p24 protein may be obtained from lysates or, preferably, be recombinantly prepared. The recombinant preparation of p24 is e.g. described in Dowbenko et al. supra, or in EP 386 713. The polypeptide may be prepared synthetically or by recombinant methods. Peptides derived from the p24 protein which can be used in the present assay are disclosed in WO 86/06414 on pages 7 and 8 or in WO 86/02383 on page 24.

Moreover this second antibody capture reagent is preferably used for the detecting high affinity antibodies, especially IgG, raised against epitopes in the antigenic regions of this protein. Another advantage of p24 for antibody detection is the ability of p24 to crossreact as a single protein with all presently known HIV types.

In a preferred embodiment, the antigen capture reagent is an antibody or a fragment thereof specific for an epitope on the p17 or p7 protein, with p7 beeing most preferred,

Accordingly, in a preferred embodiment an HIV-1 virus is detected by the assay of the present invention wherein

a) the antigen capture reagent is an antibody or a fragment thereof specific for an epitope on the p7 or p17 protein,

b) the antibody capture reagent specific for the envelope antibody analyte is a

peptide or polypeptide comprising the aminoacid sequence CSGKLIC from gp41 or a variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic antigen and

c) the antibody capture reagent specific for the capsid antibody analyte is a peptide or polypeptide comprising an aminoacid sequence corresponding to an epitope specific for the p24 protein.

When the detection of the antibody analytes is performed in a sandwich format, the indicator reagents for the antibody analytes, which bind to an epitope on gp41 or p24, have the same epitope specificity as the corresponding antibody capture reagents and, therefore, comprise the same epitope as the capture reagent. The indicator reagent is labelled or can be labelled with an enzyme. Preferably, the enzyme be horseradish peroxidase (HRPO), Other labels are acridinium ester or a ruthenium complex.

Accordingly, the analytes may be detected in a sandwich immunoassay by contacting the analytes with

a) at least one antigen indicator reagent which is an antibody or a fragment thereof specific for an epitope in the p7 or p17 antigen analyte, and

b) at least two antibody indicator reagents wherein

each of the antibody indicator reagents has the same epitope specificity as the corresponding antibody capture reagent mentioned in the paragraph before. This means for, e.g., the envelope antibody analyte that the antibody indicator reagent comprises the aminoacid sequence CSGKLIC from gp41 or a variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic antigen.

The assay can also be used for the detection of HIV-2. This virus and its antigens have been described by Guyader at al. (Nature 326 (1987) 662-669) or in more detail in WO 87/04459 and can be obtained according to the teaching of this references. The size of the corresponding proteins is in some cases slightly different from those of HIV-1. Accordingly, HIV-2 gp36 corresponds to HIV-1 gp41 and the HIV-1 capsid protein of HIV-2 is p26.

For use in the present invention, the antigen capture antibody preferably is an antibody against an antigen derived from the HIV-2 gag proteins p16 (MA), p2, p8 (NC), p1 or p6. One antibody capture reagent comprises an epitope which is specific for the envelope antibody analyte. In particular, the capture reagent comprises an epitope which is specific for the HIV-2 envelope gp140 or gp36 protein. Preferably, the capture reagent is

derived from gp36 and is a polypeptide or peptide which comprises the aminoacid sequence CAFRQVC in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form. This sequence corresponds to aminoacids 597-603 according to Guyader et al. (Nature, 326, 662-669 (1987). This capture reagent is used preferably for the detection of low affinity antibodies. Peptides which can be used as capture reagents and as indicator reagents in case of a sandwich immunoassay are described EP 326 490 A2. In particular peptides of formula (II) described on page 5, line 37 - page 6, line 15 and claimed in claim 8 of said application can be used for that purpose. These capture reagents are preferably used to detect low affinity antibodies, in particular IgM.

The second capture reagent for the capsid antibody analyte comprises an epitope which is specific for the HIV-2 core p26 protein. HIV-2 peptides derived from the p26 are described in EP 284 383. This capture reagent is preferably used for detecting high affinity antibodies, in particular IgG. Moreover, for the detection of an HIV-1 and/or an HIV-2 the antibody capture reagent can be either derived from the HIV-1 p24 or HIV-2 p26 protein since these two antigenic proteins are highly crossreactive.

According to the parameters given above, a virus belonging to the group of HIV-2 may be detected in accordance with the assay of the present invention wherein

a) at least one antigen capture reagent is an antibody or a fragment thereof which is specific for an epitope on the HIV-2 gag proteins p16, p2, p7, p1, or p6,

b) at least two antibody capture reagents wherein one antibody capture reagent comprises an epitope which is specific for the HIV-2 envelope gp140 or gp36 protein, and

the antibody capture reagent for the capsid antibody analyte comprises an epitope which is specific for the HIV-2 p26 protein.

When the detection of the HIV- 2 antibody analytes is performed in a sandwich format, the indicator reagents for the antibody analytes, which binds to an epitope on gp36 or p26, have the same epitope specificity as the corresponding antibody capture reagents and, therefore, comprises the same epitope as the capture reagent. The antigen indicator reagent is an antibody or a fragment thereof specific for an epitope in the p16, p2, p7, p1, or p6 antigen analyte

As already outlined above, it is contemplated and within the scope of the invention that the simultaneous detection of HIV-1 and HIV-2 antigen and antibody analytes is possible with the assay of the invention by adding further capture reagents for HIV-2 antigen and/or antibody analytes to the HIV-1 assay.

In one embodiment, an HIV-2 specific antigen capture reagent and two specific antibody capture reagents mentioned above may be used which can be attached to a solid support in addition to the previously described HIV-1 antigen and antibody capture reagents. The solid support for the HIV-2 antigen and/or antibody capture reagents can be the same solid support to which all other capture reagents are attached. Alternatively, a solid support may be used to which all antigen capture reagents are attached or can be attached and a solid support to which all antibody capture reagents can be attached. In another embodiment, a solid support may be used to which each capture reagent is or can be attached separately. The assay procedure would be the same as described hereinabove for the various embodiments of the invention.

In another embodiment, the addition of an envelope antibody capture reagent only already improves the detection of the antibodies raised against these antigens. Preferably, one HIV-2 env antibody capture reagent is added for detecting HIV-2 besides HIV-1 simultaneously. This addition may be done with or without using a crossreactive antibody capture reagent in the assay of the present invention as outlined below.

Additionally, by choosing HIV-1 and HIV-2 antigen capture reagents or by choosing HIV-1 or HIV-2 antigen capture reagents, the assay can be adjusted as to wether HIV-1 or HIV-2 antigens or both will be detected. For example, HIV-1 antigens may not be detected because the assay is preferably adjusted to detect HIV-2 gag antigen analytes and envelope and capsid antibody analytes together with HIV-1 envelope and capsid antibody analytes.

In combining the various embodiments, the assay consists of simultaneously or sequentially contacting the sample with
a) at least one antigen capture reagent which is an antibody or a fragment thereof specific for an epitope on an HIV-1 gag antigen analyte and/or one antigen capture reagent which is an antibody or a fragment thereof specific for an epitope on an HIV-2 gag antigen analyte,

b) two antibody capture reagents wherein
one antibody capture reagent comprises an epitope which is specific for an HIV-1 envelope antibody analyte,
one antibody capture reagent comprises an epitope which is specific for an HIV-1 capsid antibody analyte; and
c) one antibody capture reagent comprises an epitope which is specific for an HIV-2 envelope antibody analyte; and
d) detecting the antigen/antigen capture reagent and antibody/antibody capture reagent complexes,
provided that the gag antigen analyte is not the capsid protein.

As mentioned above, an antibody capture reagent comprising an epitope which is specific for an HIV-2 capsid antibody analyte may also be added or used alone in the assay described in the paragraph before. Accordingly, in part b) an antibody capture reagent specific for an HIV-1 capsid antibody and/or an antibody capture reagent specific for an HIV-2 capsid antibody may be used. In a preferred embodiment, an antigen capture reagent for an HIV-1 gag antigen analyte is used in a) and a capture reagent for an HIV-1 capsid antibody is used in b).

In another embodiment, an antibody capture reagent derived from a capsid antigen is utilized in step b) wherein the antibody capture reagent for the capsid antibody analyte crossreacts with an antibody analyte for the capsid antigen of another HIV. In this case the addition of a second capsid capture reagent is not necessary. Preferably, this capture reagent is used for detecting high affinity antibodies. Such an assay may be useful as a screening assay for HIV-1 and HIV-2.

In a particular preferred embodiment, an HIV-1 gag antigen capture reagent is used together with an antibody capture reagent for the capsid antibody analytes which is specific for an HIV-1 and an HIV-2 capsid antibody analyte. This means that the antibody capture reagent in b) specific for the HIV-1 capsid antibody analyte crossreacts with an HIV-2 capsid antibody analyte. In particular, it crossreacts with antibodies raised against p24 of HIV-1 and p26 of HIV-2. Such an antibody recognizes an epitope on the p24 HIV-1 protein and also recognizes a corresponding epitope on the p26 HIV-2 protein. A suitable antibody capture reagent specific for the capsid antibody analyte is the substantially complete p24 or p26 protein.

Accordingly, such an assay comprises in this specific embodiment the steps of simultaneously or sequentially contacting the sample with capture reagents wherein

a) the antigen capture reagent is an antibody or a fragment thereof specific for an epitope on the p7 or p17 HIV-1 protein,

b) the antibody capture reagent specific for the HIV-1 envelope antibody analyte is a peptide or polypeptide comprising the aminoacid sequence CSGKLIC from HIV-1 gp41 or a variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic antigen,

the antibody capture reagent specific for the HIV-1 p24 capsid antibody analyte is a peptide or polypeptide comprising an aminoacid sequence which crossreacts with an HIV-2 p26 antibody analyte, and

c) the antibody capture reagent for the HIV-2 envelope antibody analyte is a polypeptide or peptide which comprises the aminoacid sequence CAFRQVC from gp36 in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form.

In case the detection is performed via an sandwich immunoassay the indicator reagents are selected such that

a) the antigen analyte is detected by an antigen indicator reagent which is an antibody or a fragment thereof specific for an epitope on the gag antigen, and

b) each of the antibody analytes is detected with an antibody indicator reagent having the same epitope specificity as the corresponding antibody capture reagent.

Furthermore, other distinguishable types of HIV may also be detected by the assay of the present invention. An HIV which belongs to the group of HIV-3 is described together with its molecular organisation in WO 89/12094. Another isolate (variant) which is closely related thereto is described in EP 591 914 A2. This variant is called HIV-1 subtype O.

In this case preferred antigen capture reagents are antibodies specific for an epitope in the gag p17, p7 or p6 proteins.

One antibody capture reagent comprises an epitope which is specific for an envelope antibody analyte. Preferably this capture reagent comprises an epitope which is specific for the gp41 envelope protein. More preferred, this capture reagent comprises an epitope with the aminoacid sequence CKGKLVC (Haesevelde et al., Journal of Virology 68 (1994) 1586-1596) or a variant thereof. Such a variant is MVP-5180/91 which contains the sequence CKGKLIC in the gp41 protein. This variant is described in detail in EP

application Publ. No. 0 591 914 A2. Peptides which can be used for the detection of envelope antibody analytes are described in EP 0 591 914, especially on page 15, lines 53-57 and in EP 673 948, especially on page 7, lines 17-19. These capture reagents are preferably used for detecting low affinity antibodies.

The antibody capture reagent comprises an epitope which is specific for a capsid antibody analyte. Preferably, the capture reagent may comprise an epitope which is specific for the HIV-3 p25. This capture reagent is a preferred capture reagent for high affinity antibodies.

As for HIV-2 mentioned above, the assay may be used for detecting the presence of HIV-1, HIV-2 and HIV-3 analytes either alone or in any combination thereof simultaneously, if analytes of one or more HIV's are present in the sample, either by using individual antigen and antibody capture and indicator reagents for each HIV or, more preferred, by using capture und indicator reagents with a specificity for an analyte which different HIV's have in common. In one embodiment for detecting also HIV-3 in a sample the assay for HIV-1 and/or HIV-2 is modified such that at least an antibody capture reagent is added which comprises an epitope which is specific for an HIV-3 envelope antibody analyte.

Accordingly, for detecting analytes which are derived from any of the three HIV types the assay comprises the steps of simultaneously or sequentially contacting the sample with

a) one antigen capture reagent which is an antibody or a fragment thereof specific for an epitope in an HIV-1 gag antigen analyte,

b) two antibody capture reagents wherein

one antibody capture reagent comprises an epitope which is specific for an HIV-1 envelope antibody analyte,

one antibody capture reagent which comprises an epitope which is specific for an HIV-1 capsid antibody analyte,

c) one antibody capture reagent which comprises an epitope which is specific for an HIV-2 envelope antibody analyte,

d) one antibody capture reagent which comprises an epitope which is specific for an HIV-3 envelope antibody analyte, and

e) detecting the antigen/antigen capture reagent and antibody/antibody capture reagent complexes,

provided that the gag antigen analyte is not the p24 protein.

In a preferred embodiment the assay comprises the steps of contacting the sample with

a) an antigen capture reagent which is an antibody or a fragment thereof specific for an epitope on the HIV-1 gag proteins p17 or p7

b) an antibody capture reagent specific for the envelope antibody analyte which reagent is a polypeptide comprising the aminoacid sequence CSGKLIC or CSGKIIC from gp41 HIV-1 or any other variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form and an antibody capture reagent for the capsid antibody analyte which reagent comprises an epitope which is specific for the HIV-1 p24 protein,

c) an antibody capture reagent specific for the envelope antibody analyte which reagent is a polypeptide comprising the aminoacid sequence CAFRQVC from gp36 HIV-2 or any other variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form and

d) an antibody capture reagent specific for the envelope antibody analyte which reagent is a polypeptide comprising the aminoacid sequence CKGKLVC from gp41 HIV-3 or any other variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form.

Of course, a capture reagent for the capsid antibody analyte may be used in step b) which is known that it also crossreacts with HIV-2 p26 and HIV-3 p25 antibody analytes. Such a capture reagent can be the HIV-1 p24 protein.

For the detection in a sandwich immunoassay the sample is also contacted with

e) an antigen indicator reagent specific for the gag antigen analyte which indicator reagent is labelled or can be labelled, and

antibody indicator reagents specific for the envelope and p24 antibody analytes which indicator reagents have the same epitope specificity as the corresponding antibody capture reagents and which are labelled or can be labelled.

In general, the antibody capture reagents comprising the env gp41 or gp36 C...C sequences mentioned above can contain the cysteins (C) in their S-S cyclic (oxidized disulphide) or non cyclic (reduced SH) form or the reagent consists of a mixture of cyclic and non cyclic form. In this mixture, the reagent in the cyclic form may be a mixture of reagents wherein each reagent contains one specific variant sequence. The same can be the case for the non cyclic reagent. Therefore, the antigenic amino acid sequence in the

cyclic and/or non cyclic form may be the same or different from each other. Preferably, the capture reagent is substantially in cyclic form.

Generally, in all assays mentioned above and in line with the specific descriptions given before preferred solid supports include a magnetic or non-magnetic bead, 20-500 nm in diameter microparticles, and a well of a reaction tray, either alone or in any combination. Although the present invention preferably utilizes recombinantly produced polypeptides, it is well within the scope of the invention to utilize synthetic peptides or viral antigens from cell culture. Thus, various synthetically prepared HIV peptides, of varying length, can be used. Further, it is contemplated and within the scope of the present invention that an recombinant antigen utilized in the assay as an antibody capture as well as an indicator reagent, respectively, may be produced in two different heterologous sources like E. coli and yeast as described in EP 313 968 A2 or in EP 307 149 A2.

The invention also relates to kits for performing the assay in its various embodiments as described hereinbefore. Said kits comprise reagents, supports and other means outlined above for detecting HIV in a sample. Accordingly, these kits may be used for the detection of HIV antigen and antibody analytes of one or more HIV's if present in a sample.

In particular, the present invention provides a test kit to simultaneously detect the presence of at least one HIV antigen analyte and at least two antibody analytes of an HIV in a sample comprising
a) at least one antigen capture reagent which is an antibody or a fragment thereof specific for an epitope in a gag antigen analyte, and
b) at least two antibody capture reagents wherein
one antibody capture reagent comprises an epitope which is specific for an envelope antibody analyte and
one antibody capture reagent comprises an epitope which is specific for a capsid antibody analyte,
provided that the gag antigen analyte is not the capsid antigen.

The kit may also contain a solid support where the capture reagents are already attached or can be attached to.

Moreover, the kit may further comprise
a) at least one antigen indicator reagent specific for an epitope on the gag antigen analyte which indicator reagent is labelled or can be labelled, and

b) at least two antibody indicator reagents for the envelope and capsid antibody analytes which indicator reagents have the same epitope specificity as the corresponding antibody capture reagents and which are labelled or can be labelled.

In case the kit is used in an assay of the present invention wherein the assay is performed as an sandwich immunoassay, the indicator reagents are such that the antigen indicator reagent is an antibody or a fragment thereof specific for an epitope in that antigen analyte which epitope is different from the epitope recognized by the antigen capture reagent. The indicator reagents for the antibody analytes have the same specificity as the corresponding antibody capture reagents.

In a preferred embodiment the test kit for detecting HIV-1 comprises
a) an antigen capture reagent which is an antibody or a fragment thereof specific for an epitope on the HIV-1 gag proteins p17 or p7
b) an antibody capture reagent specific for the envelope antibody analyte which reagent is a polypeptide comprising the aminoacid sequence CSGKLIC or CSGKIIC from gp41 HIV-1 or any other variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form and an antibody capture reagent for the capsid antibody analyte which reagent is a polypeptide comprising an epitope which is specific for the HIV-1 p24 protein.

Moreover, the test kit may further comprise
a) an antigen indicator reagent which is an antibody or a fragment thereof specific for an epitope in one of the HIV-1 gag proteins p17 or p7, and
b) antibody indicator reagents for each of the gp41 envelope and p24 antibody analytes having the same specificity as the corresponding antibody capture reagents,
wherein all indicator reagents are labelled or can be labelled.

In a more preferred configuration the test comprises reagents wherein
a) the antigen capture and indicator reagents are a pair of anti-p7 antibodies,
b) the capture and indicator reagent for the envelope antibody analyte is recombinant gp41, and
the capture and indicator reagent for the p24 antibody analyte is recombinant p24,
wherein all indicator reagents are labelled or can be labelled.

For the additional detection of HIV-2 and/or HIV-3 antibody analytes, the test kit may further comprise

c) an antibody capture reagent specific for an envelope antibody analyte which reagent is a polypeptide comprising the aminoacid sequence CAFRQVC from gp36 HIV-2 or any other variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form and/or

d) an antibody capture reagent specific for an envelope antibody analyte which reagent is a polypeptide comprising the aminoacid sequence CKGKLVC from gp41 HIV-3 or any other variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form, and

e) antibody indicator reagents having the same epitope specificity for the envelope antibody analytes as the antibody capture reagents which reagents are labelled or can be labelled.

Accordingly, for detecting HIV-1, HIV-2 and HIV-3 the kit comprises

a) an antigen capture reagent which is an antibody or a fragment thereof specific for an epitope on the HIV-1 gag proteins p17 or p7

b) an antibody capture reagent specific for the envelope antibody analyte which reagent is a polypeptide comprising the aminoacid sequence CSGKLIC or CSGKIIC from gp41 HIV-1 or any other variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form and an antibody capture reagent for the capsid antibody analyte which reagent comprises an epitope which is specific for the HIV-1 p24 protein,

c) an antibody capture reagent specific for the HIV-2 envelope antibody analyte which reagent is a polypeptide comprising the aminoacid sequence CAFRQVC from gp36 HIV-2 or any other variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form and

d) an antibody capture reagent specific for the HIV-3 envelope antibody analyte which reagent is a polypeptide comprising the aminoacid sequence CKGKLVC from gp41 HIV-3 or any other variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form, and

e) an antigen indicator reagent specific for the gag antigen analyte which indicator reagent is labelled or can be labelled, and

antibody indicator reagents having the same epitope specificity for the envelope and p24 antibody analytes as the antibody capture reagents which reagents are labelled or can be labelled.

Finally, the test kits may further comprise means for labeling and/or detecting the indicator reagents.

The present invention will now be further described by some Examples according to which the invention may be performed. However, any other procedure known to a person skilled in the art and as described in the references cited above can be applied for obtaining and using the antigens and antibodies as reagents in the assay of the present invention.

Examples

The assay of the present invention can be performed according to the teaching given above. The following Examples are provided for showing how to proceed in detecting antigen and antibody analytes of several distinct HIV's, if present in a sample.

Example 1: Bead Coating and MAb-POD-conjugation

Anti p7-antibodies are coated with several HIV antigens to 1/4 inch polystyrene beads at a concentration of 1 µg/ml in a 50 mM carbonate buffer (pH 9.0) overnight at room temperature. Then 2 µg/ml HIV-1 rec. p24, 2 µg/ml HIV-1 gp41 rec. env protein, and optionally 1 µg/ml HIV-2 gp36 rec. env protein and/or 0.5 µg/ml HIV-3 env gp41 protein are simultaneously coated at pH 4.0 in 100 mM $Na_2H\ PO_4/NaH_2PO_4$, 1 mM EDTA, 0.25 % Nonidet NP40 for 12 hours at 4 °C. The beads are washed with 100 mM $Na_2H\ PO_4/NaH_2PO_4$, 50 mM NaCl pH 7.0, 0,1 % Tween 20 and blocked with 0.1% BSA, 1.5% sucrose, 100 mM $Na_2H\ PO_4$, 50 mM NaCl pH 7.0 and dried for 24 hours at 37° C in a dry air climate chamber (rel. humidity 10 - 15%).

The anti-p7 monoclonal antibodies are conjugated to horse radish peroxidase (POD), according to the methode of Nakane and Kuwaori (J. Histochem. Cytochem. 22 (1974) 1084).

Example 2: Assay for the simultaneous detection of HIV antigen and antibodies.

Tests can be performed, e.g., with the semiautomated Cobas EIA system (F. HOFFMANN-LA ROCHE Ltd.) The beads are incubated for 3 hours with 50 µl specimen diluent which contains Triton X-100® (Polyethylenglycol tert-octylphenylether, Fluka, Buchs, Switzerland), 0,05% Tween 20® (Polyoxyethylensorbitanmonolaurat, Merck-Schuchardt, Munich, Germany) and 150 µl of sample at 37° C in Cobas EIA test tubes. The beads are washed and subsequently incubated for 2.5 hours with 200 µl conjugate working solution comprising HIV1 gp41-POD, HIV1 p24-POD and

MAb anti-HIV1 p7-POD conjugate. An HIV2 gp36-POD and/or an HIV3 gp41-POD conjugate can optionally also be added depending on the beads prepared in Example 1. The beads in the test tubes are washed using the automated Cobas EIA washer. 250 µl of substrate working solution is added to the test tubes for 15 minutes. The enzymatic peroxidase reaction is stopped after addition of 1 ml stopping solution (sulphoric acid). Within one hour of stopping the enzymatic reaction, the absorbance is measured at 450 nm.

Claims

1. An assay to simultaneously detect the presence of antigen and antibody analytes of a human immunedeficiency virus (HIV) in a sample, comprising the steps of
simultaneously or sequentially contacting the sample with
a) at least one antigen capture reagent which is an antibody or a fragment thereof specific for an epitope on a gag antigen analyte,
b) at least two antibody capture reagents wherein
one antibody capture reagent comprises an epitope which is specific for an envelope antibody analyte, and
one antibody capture reagent comprises an epitope which is specific for a capsid antibody analyte, and
c) detecting the antigen/antigen capture reagent and antibody/antibody capture reagent complexes,
provided that the gag antigen analyte is not the capsid antigen.

2. The assay according to claim 1, wherein the antigen analytes and the antibody analytes are detected by contacting the analytes with
a) at least one antigen indicator reagent which is an antibody or a fragment thereof specific for an epitope in the gag antigen analyte, and
b) with at least two antibody indicator reagents wherein
one antibody indicator reagent comprises an epitope which is specific for the envelope antibody analyte, and
one antibody indicator reagent comprises an epitope which is specific for the capsid antibody analyte,
and wherein each of the antibody indicator reagents has the same epitope specificity as the corresponding antibody capture reagent.

3. The assay according to claim 1 or claim 2, wherein the antigen analytes and the antibody analytes belong to a virus from the group of HIV-1, HIV-2 or HIV-3.

4. The assay according to any one of claims 1-3, wherein one gag antigen analyte is detected and one antibody analyte for an envelope antigen and one antibody analyte for the capsid antigen are detected.

5. The assay according to any one of claims 1-4, wherein the capture reagent for the capsid antibody analyte crossreacts with an antibody analyte for the capsid antigen of another HIV.

6. The assay according to any one of claims 1-5, wherein additional antigen and/or antibody capture reagents specific for a gag antigen or envelope antibody analyte of another HIV are added.

7. The assay according to any one of claims claim 1-6, wherein the antibody analyte specific for the envelope antigen is a low affinity antibody, and
the antibody analyte specific for the capsid antigen is a high affinity antibody.

8. The assay according to claim 7, wherein the low affinity antibody is IgM and the high affinity antibody is IgG, IgA and/or IgE.

9. The assay according to any one of claims 1-6, wherein the antigen capture reagents and the antibody capture reagents are bound or can be bound to the same support or to different supports.

10. The assay according to claim 9, wherein
a) one antigen capture reagent is bound or can be bound to a first support and
b) the two antibody capture reagents specific for the envelope and the capsid antibody analytes are bound or can be bound independently either alone or in any combination to the first and/or to a second support or to a second and/or a third support.

11. The assay according to claim 9 or claim 10, wherein
a) the antibody capture reagent specific for the envelope antibody analyte is bound to a support or can be bound to a support in a density for binding and detecting a low affinity antibody, and
b) the antibody capture reagent for the capsid antibody analyte is bound to a support or can be bound to a support in a density for binding and detecting a high affinity antibody.

12. The assay according to claim 1, wherein the virus is from the group of HIV-1 and
a) one antigen capture reagent is an antibody or a fragment thereof which is specific for an epitope on the HIV-1 gag proteins p17, p2, p7, p1, or p6,
b) one antibody capture reagent for the envelope antibody analyte comprises an epitope which is specific for the HIV-1 envelope gp160 or gp41 protein, and

one antibody capture reagent for the capsid antibody analyte comprises an epitope which is specific for the HIV-1 p24 protein.

13. The assay according to claim 12, wherein the antibody capture reagent for the envelope antibody analyte is a peptide or polypeptide comprising the aminoacid sequence CSGKLIC or CSGKIIC from gp41 or a variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form.

14. The assay according to claim 12 or claim 13, wherein the antigen capture reagent is an antibody or a fragment thereof specific for an epitope on the p17 or p7 protein,

15. The assay according to claim 6, wherein the assay comprises the steps of simultaneously or sequentially contacting the sample with
a) at least one antigen capture reagent which is an antibody or a fragment thereof specific for an epitope on an HIV-1 gag antigen and/or one antigen capture reagent which is an antibody or a fragment thereof specific for an epitope on an HIV-2 gag antigen analyte,
b) two antibody capture reagents wherein
one antibody capture reagent comprises an epitope which is specific for an HIV-1 envelope antibody analyte,
one antibody capture reagent comprises an epitope which is specific for an HIV-1 capsid antibody analyte, and
c) one antibody capture reagent comprises an epitope which is specific for an HIV-2 envelope antibody analyte, and
d) detecting the antigen/antigen capture reagent and antibody/antibody capture reagent complexes,
provided that the gag antigen analyte is not the capsid antigen.

16. The assay according to claim 15, wherein the antibody capture reagent specific for an HIV-1 capsid antibody analyte crossreacts with an HIV-2 capsid antibody analyte.

17. The assay according to claim 15 or claim 16, wherein an antibody capture reagent is added which comprises an epitope which is specific for an HIV-3 envelope antibody analyte.

18. The assay according to any one of claims 12-17, wherein
a) the antigen analyte is detected by an antigen indicator reagent which is an antibody or a fragment thereof specific for an epitope on the gag antigen, and

b) each of the antibody analytes is detected with an antibody indicator reagent having the same epitope specificity as the corresponding antibody capture reagent.

19. A test kit to simultaneously detect the presence of at least one HIV antigen analyte and at least two antibody analytes of an HIV in a sample comprising
a) at least one antigen capture reagent which is an antibody or a fragment thereof specific for an epitope in a gag antigen analyte, and
b) at least two antibody capture reagents wherein
one antibody capture reagent comprises an epitope which is specific for an envelope antibody analyte and
one antibody capture reagent comprises an epitope which is specific for a capsid antibody analyte,
provided that the gag antigen analyte is not the capsid antigen.

20. The test kit according to claim 19, which further comprises
a) at least one antigen indicator reagent specific for an epitope on the gag antigen analyte which indicator reagent is labelled or can be labelled, and
b) at least two antibody indicator reagents for the envelope and capsid antibody analytes which reagents have the same epitope specificity as the corresponding antibody capture reagents and which are labelled or can be labelled.

21. The test kit according to claim 18 or claim 20 for detecting antigen and antibody analytes of an HIV-1, comprising
a) an antigen capture reagent which is an antibody or a fragment thereof specific for an epitope on the HIV-1 gag proteins p17 or p7
b) an antibody capture reagent specific for the envelope antibody analyte which reagent is a polypeptide comprising the aminoacid sequence CSGKLIC or CSGKIIC from gp41 HIV-1 or any other variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form and an antibody capture reagent for the capsid antibody analyte which reagent is a polypeptide comprising an epitope which is specific for the HIV-1 p24 protein.

22. The test kit according to claim 21, which further comprises
a) an antigen indicator reagent which is an antibody or a fragment thereof specific for an epitope in one of the HIV-1 gag proteins p17 or p7, and
b) antibody indicator reagents for each of the gp41 envelope and p24 antibody

analytes having the same epitope specificity as the corresponding antibody capture reagents,
wherein all indicator reagents are labelled or can be labelled.

23. The test kit according to claim 22, wherein
a) the antigen capture and indicator reagents are a pair of anti-p7 antibodies or fragments thereof,
b) the capture and indicator reagent for the envelope antibody analyte is recombinant gp41, and
the capture and indicator reagent for the capsid antibody analyte is recombinant p24,
wherein all indicator reagents are labelled or can be labelled.

24. The test kit according to any one of claims 21-23, which further comprises
c) an antibody capture reagent specific for an HIV-2 envelope antibody analyte which reagent is a polypeptide comprising the aminoacid sequence CAFRQVC from gp36 HIV-2 or any other variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form and/or
d) an antibody capture reagent specific for an HIV-3 envelope antibody analyte which reagent is a polypeptide comprising the aminoacid sequence CKGKLVC from gp41 HIV-3 or any other variant thereof in its S-S cyclic or non cyclic form or as a mixture of cyclic and non cyclic form, and
e) antibody indicator reagents having the same epitope specificity for the envelope antibody analytes as the antibody capture reagents which indicator reagents are labelled or can be labelled.

25. The test kit according to any one of claims 21-24, which further comprises means for labeling and/or detecting the indicator reagents.

26. Use of a test kit according to any one of claims 19-25 for the detection of HIV antigen and antibody analytes in a sample.

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